The Evaluation of Uncertainty for the Measurement of Blood Ethanol

Kan Etanol Ölçümü için Belirsizliğin Değerlendirilmesi

Uncertainty in the Measurement of Blood Ethanol

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Özet

Amaç: Adli tıpta, etanol düzeylerinin ölçülmesi bireylere karşı açılan davalarda önemlidir. Her ölçüm sonucu belli bir miktar belirsizliğe sahiptir. Bu çalışmanın amacı, alkol dehidrojenaz yöntemi kullanılarak yapılan etanol ölçümünde belirsizliği belirlemek, aynı zamanda değişkenliğin kaynaklarını ve bunların belirsizliğe olan katkılarını değerlendirmektir. Gereç ve Yöntem: Etanol için tekrarlanabilirlik, kalibratör belirsizliği ve stabilite belirsizliği belirlendi. Bunların standart belirsizlikleri hesaplandıktan sonra her bir değerin kareleri toplandı ve bu değerin kare kökleri alınarak kombine standart belirsizlik değeri bulundu. Genişletilmiş belirsizlik, kombine standart belirsizlik değerinin kapsam faktörü (k=2, %95 güven aralığı) ile çarpımından elde edildi. Bulgular: Etanol için ölçüm belirsizliği, %8.5 (%95 güven aralığında) olarak saptandı. Eşik değerdeki sonuç (50 mg/dL), 50±4.3 mg/dL (%95 güven aralığında) olarak rapor edildi. Tartışma: Alkol ölçümü yapan akredite bir laboratuvarda sonuçların doğruluğunu ve güvenilirliğini sağlamak için ölçüm belirsizliği hesaplanmalıdır. Preanalitik, analitik ve post analitik aşamalarda sonuçları etkileyebilecek hata kaynakları tespit edilmeli ve belirsizlik değeri verilmelidir.

Anahtar Kelimeler

Etanol; Belirsizlik; Alkol Dehidrojenaz

Abstract

Aim: In forensic medicine, measurement of ethanol levels is important in lawsuits filed against individuals. Each measurement result has a certain amount of uncertainty. The objective of this study is to determine uncertainty of ethanol measurement using the alcohol dehydrogenase method, and also to evaluate sources of variability and their contributions to uncertainty. Material and Method: Repeatability, calibrator uncertainty, and stability uncertainty were determined for ethanol measurement. After their standard uncertainties were calculated, the squares of each value were summed and the combined standard uncertainty value was found with the square roots of that value. The expanded uncertainty value was obtained by multiplying the combined standard uncertainty value by a coverage factor (k=2; confidence interval, 95%). Results: The uncertainty of measurement for ethanol was found to be ±8.5% (confidence interval, 95%). The result at threshold level (50 mg/dL) was reported as 50±4.3 mg/dL (confidence interval, 95%). Discussion: Measurement uncertainty should be estimated to ensure accuracy and reliability of the results in an accredited laboratory performing alcohol measurement. Sources of error that might affect the results during the pre-analytical, analytical, and post-analytical phases should be identified and the uncertainty value should be calculated.

Keywords

Ethanol; Uncertainty; Alcohol Dehydrogenase

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Introduction

Although alcohol, after coffee, is the second most commonly consumed addictive substance affecting mental and physical activity; it is also the most frequently tolerated drink after coffee [1]. Therefore, in many countries the determination of permissible blood alcohol levels as part of traffic regulations has been affected not just by scientific justifications but also by societal tendencies. In many regions of the world there are legal blood alcohol limits for drivers of motor vehicles. In compliance with the Road Traffic Act in Turkey, drivers of private taxis, taxicabs, minibuses, buses, lorries, and trailers are not allowed to drive while drunk. Private car drivers are not permitted to drive with a blood alcohol concentration greater than 50 mg/dL (according to Road Traffic Act # 2918 dated 6.16.1985 in Turkey). Governments of Austria, Denmark, France, Australia, Canada and The Netherlands have legal limits of 50 mg/dL for blood alcohol concentration for motor vehicle drivers. Limits of permissible alcohol intake vary by country (US, 50-80 mg/dL; UK, 80 mg/dL; Japan, 30 mg/dL; Sweden, 20 mg/dL; Norway, 20 mg/dL; Russia, 20 mg/dL; Poland, 30 mg/dL; Hungary and Romania, 0 mg/dL) [1].

The most practical tool for the breath test is the alcoholometer. It is the prevalent tool used for traffic control. Although this method, which has a margin of error, is not sensitive enough, it is still considered a satisfactory method to determine a driver's blood alcohol concentration. Certainly, if alcohol consumption is close to the legal limit, a blood analysis should also be performed. The main concern is that erroneous results of blood alcohol measurements mislead legal authorities. Therefore, laboratory test results should be accurate and reliable.

Measurement uncertainty is a parameter characterizing the dispersion of the values attributed to a reasonably measured quantity according to Eurachem/CITAC Guide CG 4 "Quantifying Uncertainty in Analytical Measurement" (QUAM). In other words, it demonstrates the extent to which the result represents the true value. Relevant international standards (as "Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results" prepared by National Institute of Standards and Technology and "EP29-A: Expression of Measurement Uncertainty in Laboratory Medicine" prepared by Clinical and Laboratory Standards Institute) have been included in the working procedures of many disciplines engaged in calibration and testing procedures. Measurement uncertainty is one of the general requirements demonstrating adequacy of a laboratory in compliance with ISO/IEC 17025 and ISO 15189 (prepared by International Organization for Standardization). Because of this requirement, measurement uncertainty is being used by accredited laboratories. However, to date only a few studies have been published on measurement uncertainty focused on the needs of forensic medical matters [2-6].

For threshold blood alcohol levels, calculation and reporting of measurement uncertainty by the laboratory will increase the reliability of the results, which might ease the relatively important decision-making process of legal authorities. The objective of this study is to evaluate sources of variability and to investigate their contribution to uncertainty of measurement of blood alcohol levels.

Material and Method

Blood alcohol levels were measured using the alcohol dehydrogenase method (3L36-20, MULTIGENT Ethanol Reagent, Abbott Diagnostic, GmbH & Co. KG, Germany) on Architect c8000 analysers (Abbott Diagnostic, GmbH & Co. KG, Germany).

Measurement uncertainty is classified as Type A or B according to Eurachem/CITAC Guide CG 4 "Quantifying Uncertainty in Analytical Measurement" (QUAM). Type A is an uncertainty value calculated using statistical methods, while Type B is estimated based on non-statistical methods based on values provided by the manufacturers. The uncertainty related to stability and of repeatability are considered as Type A. The uncertainty related to the calibrator is considered as Type B.

Repeatability (urep): The control samples of 50 mg/dL (3L36-10, MULTIGENT Ethanol 50 Control, Abbott Diagnostic, GmbH & Co. KG, Germany) were analyzed repetitively for 20 times (n = 20) within run, and then the coefficient of variation (CV) was estimated. To comply with the normal distribution of data, the standard uncertainty (urep) was calculated as CV of the result of 20 measurements divided by the square root of n (√n) (Type A).

urep = CV / √n

Calibrator uncertainty (ucal): The uncertainty values related to the calibrator (3L3602, MULTIGENT Ethanol 100 Cal, Abbott Diagnostic, GmbH & Co. KG, Germany) provided by the manufacturer were used. The uncertainty value of the ethanol calibrator (Cal) at a concentration of 100 mg/dL was estimated as 6.7 mg/dL.

Ccal, concentration of the calibrator; and ucer, uncertainty of the calibrator, are recorded in the certificate. Coverage factor (k) is given as k = 2, since a 95% confidence level is used (Type B).

Relative standard uncertainty (ucal) = 100 × ucer / k × Ccal

Stability uncertainty (ustab): The study group consisted of 20 drivers who drink alcohol. Written informed consent was obtained from each participant before inclusion in the study. The procedures were in accordance with the guidelines of the Helsinki Declaration on human experimentation. Blood samples collected into gel-barrier sampling vacuum tubes (Vacutainer®) were centrifuged at 3000 rpm at 25oC for 5 minutes without opening tube caps. Time interval from opening of sample tube caps to the pipetting the sample into the device is thought to be at most 30 minutes. Blood samples (n=20) with certain serum ethanol levels (50-100 mg/dL) were analyzed at baseline and again 30 minutes later. For each concentration, mean of differences% (diff%) was calculated. Assuming compatibility with rectangular distribution, the standard uncertainty was calculated dividing mean of differences by a square root of 3 (√3) (Type A).

ustab = diff% / √3

Combined standard uncertainty (uc): After all standard uncertainties were determined, the squares of each value were summed up and the combined standard uncertainty was found by taking the sum's square root.

(uc)2 = (urep)2 + (ucal)2 + (ustab)2

Expanded uncertainty (U): The value of the combined standard uncertainty was obtained by multiplying by a coverage factor (k = 2) within the 95% confidence level.

U = k × uc

Results

In our study, repeatability, calibrator, stability and expanded uncertainty values of ethanol were estimated. Standard uncertainty values and ratios are given in Table 1. In our study, the measurement uncertainty of ethanol within 95% confidence level was found as ±8.5%. The result at threshold level (50 mg/dL) was reported as x±U mg/dL, ie. 50±4.3 mg/dL.

Table 1. The uncertainty values and its ratios.

	Uncertainty value	Uncertainty budget
a. Repeatability		
urep= 4.44 / √20	0.99	15%
b. Calibrator uncertainty		
urcal= (100 × 6.7) / (2 × 100) = 3.35	3.35	50%
c. Stability uncertainty		
ustab= 4.13 / √3	2.39	35%
d. Combined standard uncertainty		
uc= √[(0.99)2 + (3.35)2 + (2.39)2]	4.23	
e. Expanded uncertainty		
U= 2 × 4.23	8.46	

Discussion

In forensic medicine, measurement of ethanol levels is important in lawsuits filed against individuals. In these cases, the method of measurement should be validated and, preferably, it should be constantly audited with quality assurance programs including internal and external quality control systems. However, uncertainty of the measurement results cannot be avoided. Therefore, when evaluating the results, uncertainty value should be taken into consideration.

One of the advantages of calculating measurement uncertainty is that it enables us to learn whether the method used has met the known performance criteria of that method. In other words, since measurement uncertainty corresponds to a total error, measurement uncertainty allows us to make evaluations based on acceptable error, which in turn provides information about the performance status of our method. Our uncertainty value (8.5%) was lower than acceptable error values (±15% and ±25%, respectively) of The College of Physicians and Surgeons of Saskatchewan and New York State Department of Health [7].

Another advantage of calculating measurement uncertainty is that the source of measurement uncertainty can be determined during the calculation process. Indeed, identification of the sources of uncertainty will lead the way to eliminate or to minimize the impact of these sources on measurement accuracy. Kristiansen et al. measured ethanol levels using the gas chromatographic method and estimated maximal measurement uncertainty as 4.95% for different blood ethanol concentrations. They suggested that mostly the analytical component (90%) and then to a lesser extent, traceability (<4%), matrix effect (<0.5%), and blood water content (<0.39%) contributed to the measurement uncertainty. They suggested that the ratio of the analytical component can be reduced by increasing the number of repeats [2]. However, Gullberg et al. indicated that a single measurement can only estimate ±20% of the actual blood alcohol concentration [6].

Fung et al. calculated total uncertainty (random and systematic error) value for measurement of blood alcohol as 4% using certified ethanol reference standards [5]. Generally, in a laboratory operating under good quality control system management, random error caused by the devices used is negligibly small in importance. Rather, errors stemming from improper use of the devices are thought to have an important impact on uncertainty. However, in a study performed by Fung et al., the authors demonstrated that deviation from the average values originating from improper use of the devices was only 0.3% without any practical significance as for uncertainty. Our results also support their studies. When we analyzed our sources of uncertainty, the greatest contribution to uncertainty is from calibrators. The second most contributing factor was impaired stability of ethanol in an open tube. This finding indicates the importance of processing ethanol in blood samples as soon as possible after opening the caps of sampling tubes to ensure reliability of the analytical result. Shorter-term studies performed at different time points may prove that the contribution of stability to uncertainty is relatively lesser and variable.

When legal consequences of the reported serum ethanol measurement results are taken into account, laboratory results should be accurate, reliable, and precise. Based on the consensus decision of the College of American Pathologists (CAP), allowable error for AxSym (Abbott Diagnostic, GmbH & Co. KG, Germany) analysers is ±80% [7]. Even the accuracy among the results of gas chromatography has been debated. Therefore, when starting to use "a standard and well-established test method" for ethanol measurement in the laboratory, measurement uncertainty should be estimated. This value will ensure comparability and reliability of the test results reported by different laboratories. Besides, during calculation of uncertainty, laboratory specialists will identify all sources of error effective on the results of the measurement and determine their impact on the results obtained.

The uncertainty value determined for ethanol tests gains special importance in the evaluation of borderline values. For example, our result reported as 50 mg/dL is above the cut-off value and seems to exceed the permissible limit, when estimated uncertainty value (8.5%) in this study is taken into consideration. In reality, it might correspond to a value between 45.7 and 54.3 mg/dL. In this case, this estimated blood alcohol level (50 mg/dL) may not definitively signify alcohol consumption of the individual above permissible legal limits. Conversely, a blood ethanol level of 48 mg/dL may not absolutely indicate that this individual has consumed alcohol below permissible legal limits.

Measurement uncertainty should be estimated to ensure accuracy and reliability of the results in an accredited laboratory performing alcohol measurement. Accordingly, legal authorities making interpretations based on these results should be informed about the subject matter, which is important for the proper progression of the legal procedure.

Competing interests

The authors declare that they have no competing interests.

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